Microenvironmental regulation of biomacromolecular therapies

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Abstract | There is currently great interest in molecular therapies to treat various diseases, and this has prompted extensive efforts to achieve target-specific and controlled delivery of bioactive macromolecules (for example, proteins, antibodies, DNA and small interfering RNA) through the design of smart drug carriers. By contrast, the influence of the microenvironment in which the target cell resides and the effect it might have on the success of biomacromolecular therapies has been under-appreciated. The extracellular matrix (ECM) component of the cellular niche may be particularly important, as many diseases and injury disrupt the normal ECM architecture, the cell adhesion to ECM, and the subsequent cellular activities. This Review will discuss the importance of the ECM and the ECM–cell interactions on the cell response to bioactive macromolecules, and suggest how this information could lead to new criteria for the design of novel drug delivery systems.

Biomacromolecule
A high molecular mass molecule formed from biological building blocks such as nucleic acids, amino acids and sugars.

Plasmid
A small circular piece of DNA that can replicate itself independently of chromosomal DNA; frequently used to introduce potentially therapeutic genes into cells.

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A broad array of therapeutic biomacromolecules including proteins[1–5], plasmid DNAs (pDNAs)[6] and various forms of RNA have been identified[7,8], engineered and used in various clinical trials and approved products[9,10]. The completion of the Human Genome Project will probably accelerate the discovery and application of biomacromolecular therapies. This new generation of biomedicines aims to prevent and treat chronic and malignant diseases, trauma, and tissue defects by altering the fate or gene expression of resident or transplanted cells.

Despite impressive successes in some diseases[11–14], biomacromolecular therapies have presented several challenges that have yet to be broadly resolved[15]. These include enzymatic degradation of the molecules, non-specific interactions with cells and limited intracellular entry of nucleotide-based therapies[16]. Tremendous efforts have been made to modify the therapeutic biomacromolecules with targeting and functional cues, and to design new delivery systems, leading to significant enhancement of therapeutic efficiency. For example, therapeutic proteins and nucleotides have been hybridized through chemical fusion or complexation with diverse targeting[17,18] and delivery molecules to enhance infiltration into tissues and cells[19,20] and tissue-specific localization[21–24]. Furthermore, therapeutic biomacromolecules have been loaded into various biomaterials to enable a sustained and localized delivery manner while preserving bioactivity, as extensively reviewed elsewhere[5,25–30].

By contrast, the importance of the microenvironment of the target cells in achieving the desired therapeutic effects has not been subject to the same scrutiny, even though the microenvironment clearly plays a critical role in the regulation of cellular behaviour[31–35]. Several studies have demonstrated that diverse activities of stem, progenitor and differentiated cells[36] are regulated by the cross-talk between cells and the extracellular matrix (ECM) through the binding interactions between integrins, a family of cell-adhesion receptors, and other receptors and adhesion ligands in the ECM[37–42] (FIG. 1a). Also, disease and trauma are often associated with an aberration of the cell–ECM interactions due to alterations in the structure and properties of the ECM[43,44] (FIG. 1b), as well as disturbance in nutrient transport and immune and inflammatory responses[45]. The ECM can be physically destroyed by mechanical loading or enzymatically eroded, leading to loss of adhesion sites, downregulation of integrin expression and destabilization of cell anchorage. Conversely, in certain diseases (for example, cancer) abnormal ECM accumulation is typical[46,47] (FIG. 1c), leading to an increase of tissue stiffness and the formation of a hypoxic environment[48]. One consequence of these changes is that the abnormal ECM may alter the response of cells to various interventions (for example, survival of malignant cells against chemotherapies and radiotherapies)[49] and alter the ability of the cell to bind growth factors and/or to take up exogenous genes and initiate the cellular machinery to activate gene expression. The disruption of the ECM in a target tissue could

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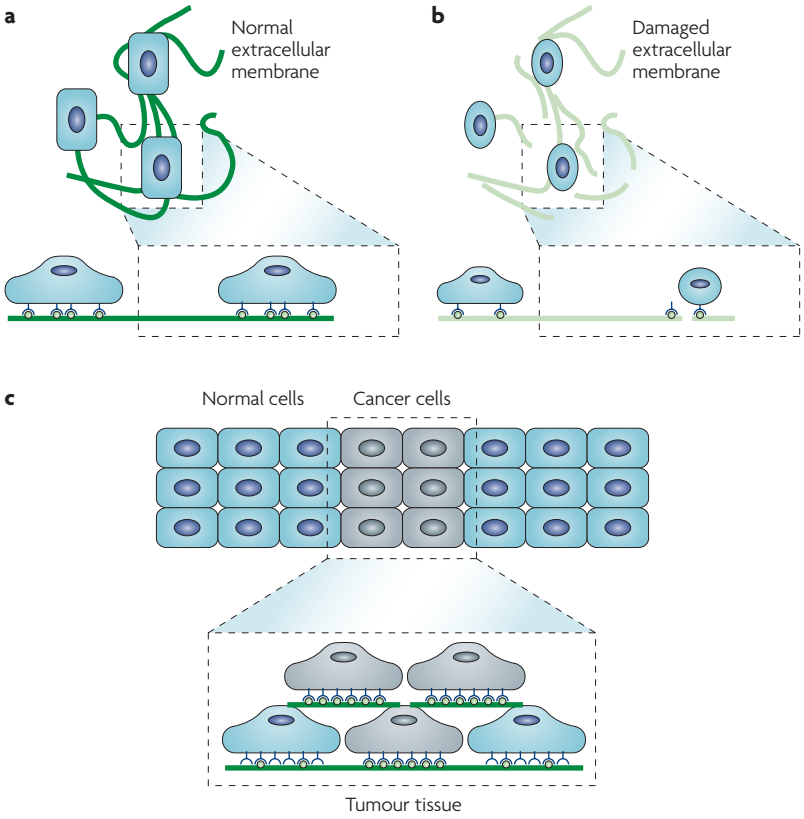

Figure 1 | **The cellular microenvironment varies with tissue injury and pathogenesis.** In a normal extracellular matrix (ECM), cells are anchored to the ECM through multiple receptor–ligand bonds (**a**). Tissue injury, which can initially reduce the stiffness of the ECM and the number of ligands, can destabilize receptor–ligand bonds, leading also to a decrease in cellular contractility (**b**). Conversely, pathological processes such as tumour formation may lead to enhanced expression of adhesion receptors, an accumulation of ECM and other changes in the microenvironment (for example, hypoxia) (**c**).

Extracellular matrix
(ECM). A complex mixture of non-living material surrounding cells within tissues. The ECM provides space for tissue formation, mechanically supports the tissue and provides cues to regulate cell function.

Integrin
A specific type of cell-surface receptor that mediates adhesion to the extracellular matrix and subsequent signalling to the cell.

Silencing RNA
20–25 nucleotide-long double-stranded RNA molecules, referred to as small interfering RNA (siRNA), used to interfere with the expression of a specific gene. siRNA treatments are also termed interference therapies.

also limit the recruitment of peripheral cells to the target tissue[50,51]. Therefore, it is perhaps not surprising that the delivery of potentially therapeutic proteins and nucleotides can result in a limited response from the target cells[44].

Significant advances in the efficiency of biomacromolecular therapies can be achieved through a greater understanding of the normal and diseased microenvironment of target cell populations, and the influence of these microenvironmental changes on the target cells. This ultimately would allow one to develop strategies to predict and amplify the cellular response to therapeutic biomacromolecules. This article first reviews data demonstrating the crucial role of microenvironmental signals in regulating the cellular response to exogenous proteins and nucleotides that are delivered for local therapies. The specific effects of the physical and chemical properties of the ECM on cellular activities, and their effects on the delivery efficiency of growth factors, antibodies, pDNA, silencing RNA (siRNA) and oligonucleotides will be discussed, as well as possible interventions to manipulate the microenvironment of target cells to improve *in vivo* efficiency of biomacromolecular therapies.

Cellular microenvironment and protein therapies

Various recombinant growth factors and antibodies are being used to elicit specific cellular activities that are useful for wound healing and regeneration of tissues and organs, or to limit the pathogenesis and metastasis of malignant cells (reviewed elsewhere)[1–5]. Different growth factors may also be combined to attain synergistic improvements in therapeutic efficiency[52,53]. Therapeutic proteins are commonly administered by tissue-specific or intravenous injection; however, an array of biodegradable matrices are being explored to allow localized and sustained delivery[27–29] while preventing the degradation of the proteins. Sequential delivery of multiple growth factors from polymeric carriers is also being exploited to achieve further enhancements in therapeutic effects[53]. However, it is not clear whether cells in varied microenvironments respond to the potentially therapeutic protein drugs in the same manner. Therefore, this section reviews the significant role of the cellular microenvironment in protein therapies (growth factors and antibodies), which specifically aim to mediate tissue morphogenesis.

The microenvironment alters the cellular response to protein therapies. Cells that are residing in target tissues suffering from disease or damage may respond differently to protein drugs compared with cells that are anchored to the normal ECM. This effect of the ECM can even be seen in developmental processes, for example, the responsiveness of mammary-gland formation to steroid signalling is mediated by the presence or alteration of adhesion molecules in the stroma[54]. Although it is difficult to definitively address this issue *in vivo*, owing to the complexity of that environment, the use of *in vitro* ECM models that mimic the chemical structure, composition, physical and chemical properties, and architecture of target tissues could allow one to predict and manipulate the therapeutic efficiency of protein therapies.

Cell adhesion and the presentation of adhesion molecules in an ECM clearly regulate the cellular response to growth factors *in vitro*. The importance of cell adhesion is exemplified by the increased level of myoblast differentiation following stimulation with transforming-growth factor (TGF) (FIG. 2); this is higher in adherent cells than suspended cells due to the activation of focal adhesion-kinase signalling[55]. Similarly, the cell-proliferation effects of epidermal growth factor (EGF) and insulin-like growth factor 1 (IGF1) are higher with cells that are adhered to substrates coated with normal ECM molecules (for example, collagen, fibronectin) than to poly-D-lysine-coated substrates[56]. This probably relates to the tensional forces that the cells exert on bioactive matrices through receptor–ligand bonds[57,58]. Furthermore, the speed of fibroblast migration stimulated by EGF is also mediated by the spatial organization of fibronectin presented from the cell-adhesion substrate[59,60]. Similarly, the efficiency of vascular endothelial growth factor (VEGF), which stimulates the formation of endothelial-cell colonies, was also enhanced in cells that were adhered to substrates coupled with cell-adhesion oligopeptides containing an Arg-Gly-Asp (RGD)

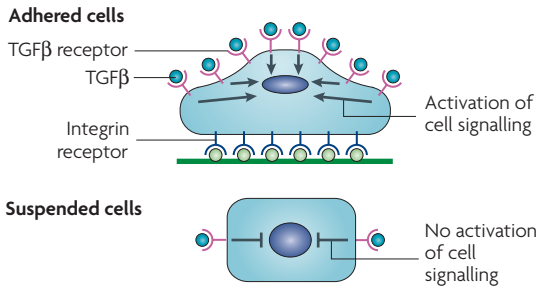

Figure 2 | ***In vitro* cell response to protein drugs is modulated by the microenvironment.** Transforming-growth factor-β (TGFβ) stimulated the differentiation of myofibroblasts adhered to an extracellular matrix, whereas it failed to stimulate those suspended in cell-culture medium[55].

sequence[61]. Conversely, cell adhesion can impair the efficiency of certain proteins. For example, endothelial cells adherent to collagen I and fibronectin demonstrate enhanced survival and stimulation of autocrine secretion of VEGF, and this reduces the efficiency of some angiostatic molecules (for example, thrombospondin, interferon-inducing protein and endostatin) that are intended to induce cell apoptosis[62]. The composition of the ECM is also important. For example, the ability of TGFβ1 to inhibit cell proliferation is higher in cells that are cultured in collagen matrices than those cultured in more complex cell-derived ECMs[63]. Human umbilical vascular endothelial cells cultured in collagen gels or Matrigel organize to form vacuoles and a lattice in the presence of basic fibroblast growth factor (bFGF; also known as FGF2), whereas those in fibrin gels did not respond to these growth factors[64]. Although, so far, it has not been systematically examined, the mechanical properties of the ECM and the microscale and nanoscale organization of cell-adhesion molecules and their total numbers in the ECM are also likely to be critical. All of these variables regulate cell proliferation, viability and differentiation[65–67]. The role of the micropattern and nanopattern of adhesion molecules can be readily probed using model cell-adhesion proteins consisting of synthetic cell-adhesion oligopeptides that are chemically coupled to non-adherent materials[67].

Manipulation of cellular receptor levels and ECM growth-factor-binding interactions also alter cell responsiveness to protein drugs. Immobilization of several growth factors including EGF and VEGF to cell-adhesion substrates has been demonstrated to mediate the growth and differentiation of cells[68,69]. The presence of supplemental binding molecules, for example heparin, can enhance the *in vitro* tube formation by endothelial cells stimulated by bFGF through the overexpression of integrin α6 (REF. 70). Complexes of fibronectin and VEGF stimulate intracellular associations between VEGF receptors (VEGFRs) and the fibronectin receptor integrin αvβ1 and subsequently enhance cell migration[71]. Conversely, disruption of αvβ3 ligation blocks angiogenesis even in the presence of several angiogenetic factors, including bFGF and tumour-necrosis factor (TNF)[72,73].

Cells cultured in three-dimensional (3D) microenvironments might present a different response to protein drugs compared with cells cultured on 2D substrates. The architecture of the ECM influences cell phenotype in multiple ways. For example, encapsulation of cells in enzymatically labile natural gels, such as Matrigel, fibrin gel and collagen gel, facilitates the formation of capillary sprouts in the presence of VEGF and anisotropic extension of neurites in the presence of nerve growth factor (NGF), unlike cells in 2D culture[74,75]. Synthetic ECM analogues could also allow a similar control[27]. Cancer cells that are cultured in a 3D microenvironment (for example, multicellular spheroids and multicellular layers) also respond differently to therapeutic antibodies (for example, apoptosis-inducing antibodies, VEGFR) compared with cells cultured on a 2D substrate, because of alterations in integrin expression[76], increased cell–cell contacts[77], varied interdependency between cell anchorage and growth factor[78], and limited penetration of antibodies in tumour-like tissues[79]. These findings in 2D and 3D cell-culture conditions are being translated to improve *in vivo* therapeutic efficiency of proteins, which are described in the next section.

Strategies to engineer the cell microenvironment and to manipulate cell responsiveness. The studies summarized in the previous section suggest that at least three strategies could be pursued to regulate the cellular response to protein therapies, including modulating cell viability in the target tissue, modulating the interaction between cells and the ECM and providing cells with an artificial microenvironment designed to provoke the desired response. Maintaining cell viability in tissues that are subjected to reversible injury is perhaps the most direct manner to provide cell responsiveness to protein drugs. Combined delivery of survival cytokines, including stem-cell factor (SCF; also known as KITLG), interleukin 3 (IL3), stromal-derived factor and thrombopoietin, have been shown to prevent the apoptosis of stem cells and progenitor cells in tissues that have been damaged by nuclear irradiation[80] (FIG. 3a). This result implies that simultaneous and/or sequential delivery of these anti-apoptotic cytokines with growth factors used to stimulate tissue regeneration could enhance tissue recovery.

Interactions between cells and the ECM can also be modulated to regulate the responsiveness of cells to protein drugs. Studies of an anti-angiogenesis therapy demonstrated that the combined delivery of an antibody to platelet-derived growth factor (PDGF) receptor, which blocked mural cell migration to endothelial tubes, and an anti-VEGF aptamer led to more effective regression of blood vessels compared to treatment with the anti-VEGF aptamer alone[81,82] (FIG. 3b). As illustrated with *in vitro* studies reviewed previously, the inverse approach of promoting cell adhesion to the ECM by upregulating integrin expression could provide an effective target to stimulate tissue regeneration and wound healing.

Introduction of a synthetic ECM is increasingly being used to enhance the therapeutic efficiency of proteins, specifically in tissue regeneration and wound-healing applications. Biomaterial-based devices, within which

Aptamer
Short nucleic-acid- or amino-acid-based molecules that bind a specific target molecule. They are often used as macromolecular drugs.

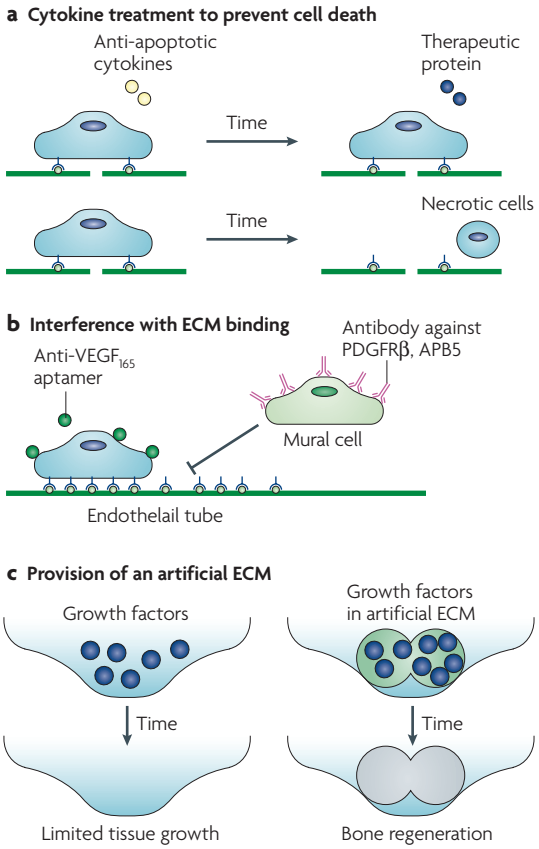

Figure 3 | **Potential strategies to engineer cell microenvironments *in vivo* to modulate the cellular response to protein drugs. a** | Delivery of anti-apoptotic cytokines may maintain cell viability following reversible injury and allow cells to subsequently respond to a therapeutic protein, whereas cells that are not exposed to the cytokines may become necrotic. **b** | Interference with cell–extracellular matrix (ECM) interactions, for example, combined delivery of an antibody (APB5) against the platelet-derived growth factor (PDGF) receptor-β, which blocked mural cell migration, and an anti-$VEGF_{165}$-aptamer effectively led to the regression of blood vessels[81,82]. **c** | Implantation of a new synthetic ECM that has embedded growth factors supports the migration of cells into the defect and regeneration of tissue (for example bone in a calvarial defect)[89]. VEGF, vascular endothelial growth factor.

growth factors are physically embedded, sequestered or immobilized, are often modified with cell-adhesion molecules in order to maximize the population of cells stimulated with growth factors. Modification of poly-(lactic acid) devices that release bone morphogenetic protein 2 (BMP2)[83] and chitosan enhanced bone regeneration due to an increase in cell adhesion to the polymer matrix. Furthermore, coupling oligopeptides that contain the RGD sequence to protein delivery vehicles may also improve the cellular response to growth factors by facilitating cell migration into the material, activating the cells to the desired responsive state and subsequently exposing the cells to the drug in a controlled manner[84]. For example, binding BMP2 to fibrin gels has stimulated the growth of bone into gels in several animal models[85]. In a similar manner, binding of VEGF and RGD-containing oligopeptides to synthetic hydrogels have also stimulated vascularization of the gels[86].

Synthetic ECM can either recruit peripheral cells and facilitate their migration into the ECM or directly transplant cells to a desired location. For example, BMP2 releasing synthetic ECMs, which present cell adhesion cues and are labile to matrix metalloproteinase (MMP), demonstrate enhanced regeneration of bone in calvarial defects compared with non-degrading ECMs[87] (FIG. 3c). Providing a biomineralized coating on synthetic polymeric protein delivery vehicles also enhanced the infiltration of blood vessels in the matrix and subsequent bone regeneration[88,89]. Bioactive growth-factor-releasing matrices can also be used as a cell transplantation vehicle. For example, keratinocytes and EGF encapsulated into a fibrin matrix stimulated epidermal regeneration in skin wound-healing[90], and hydrogels containing bone-forming cells, bFGF and BMP2 improved bone regeneration[91–93]. A more refined presentation of cell-adhesion cues in the materials using nanoscale and microscale patterning techniques[94–96] could provide an even greater level of control and improve the therapeutic efficiency of proteins both when host cells are recruited into the target tissue and when cells are transplanted.

Cellular niche and nucleotide therapies

Nucleotide therapies have been proposed for the treatment of metabolic deficiency (for example, family cholesterolaemia)[97]; neurodegenerative diseases[98]; infectious diseases (for example, HIV[99]); immunization (for example, cancer vaccines[100]); and tissue regeneration (for example, bone, muscle, skin, blood vessels[26,101]). In principle, nucleotides that contain a sequence encoding for specific proteins or hormones can be introduced into target cells and the cell machinery can be exploited to produce the desired proteins. Alternatively, nucleotides that contain a sequence complementary to a specific gene or mRNA can be used to trigger their degradation and return abnormal gene expression to a desired state in approaches such as antisense therapies and interference therapies (siRNA)[102,103]. These nucleotide therapies can potentially alter cellular activities over longer periods of time compared with protein therapies. The success of nucleotide therapies depends on the efficiency of their passage through the cell membrane and subsequent activation or deactivation of target gene expression. Extensive efforts have been made to enhance the efficiency of pDNA[104] and siRNA therapies[105] as non-viral delivery vectors are increasingly being used because of the safety concerns related to the immunogenicity of viral vectors. These efforts comprise complexation with an array of synthetic delivery molecules, including positively charged lipids, polymers, dendrimers and nano-sized particles and wires; encapsulation of pDNA into artificial virus particles[19,21]; and use of several physical stimulatory tools[106,107]. However, these efforts typically attempt to manipulate the chemical and/or physical state of the nucleotide drugs, irrespective of the cell microenvironment; here, we will discuss the roles of the microenvironment in mediating the cellular response to nucleotides.

Matrix metalloproteinase
(MMP). A specific type of enzyme that is capable of degrading extracellular-matrix proteins.

Antisense therapy
A treatment that uses a single strand of nucleic acid (antisense oligonucleotide) that is designed to bind a specific mRNA and inactivate its expression.

The microenvironment alters the cellular response to nucleotide therapies. Efficient delivery of therapeutic nucleotides must overcome several biochemical and biophysical barriers, which can include passage across the cell membrane, escape from endosomes, transport through the cytosol and across the nuclear membrane. Plasmid DNA therapies must then be transcribed, whereas oligonucleotides and siRNAs inhibit specific gene-expression processes. The ability of resident cells in target tissues that are suffering from disease or damage to take up these nucleotides and activate the appropriate cell machinery is likely to be quite distinct from the response of cells anchored to a normal ECM in a disease or damage-free situation. The complexity of the *in vivo* microenvironment has discouraged direct studies on this issue, but more extensive *in vitro* studies with ECM models have elucidated some of the important issues. The presentation of cell-adhesion molecules, architecture of the ECM and its physical properties, and external mechanical stimulation can all regulate the cellular response to exogenous nucleotides as they alter the overall cell phenotype.

Cell-adhesion ligands modulate the efficiency of gene transfer and subsequent gene expression level, and at least some of these effects are regulated to control mitosis, which temporally disrupts the cell and nuclear membrane[108,109]. Fibronectin or fibronectin fragments, when physically or chemically coupled to cell-adhesion substrates, have been demonstrated to enhance gene expression following pDNA exposure through their promotion of cell proliferation. This effect of fibronectin has also been found with delivery of retroviral gene vectors[110–113]. Synthetic oligopeptides that contain the adhesion site of fibronectin (the RGD sequence) have been chemically coupled to adhesion substrates to provide a simple model system to address this issue, and the nanoscale distribution of these adhesion oligopeptides was found to mediate the efficiency of gene delivery[114]. Raising the density of oligopeptides (N_{RGD}) greatly enhanced the ability of the cells to take up pDNA and subsequently increased the gene expression level, whereas increasing the spacing between clusters of oligopeptides on the nanometer scale (d_{RGD}) limited the ability of cells to take up pDNA (FIG. 4a,b). These effects correlated to control by the adhesion peptides over the frequency of cellular multiplication. The potential relationship between these effects and the intracellular transport of pDNA and activation of cell machinery that regulate gene expression remain to be examined.

The ECM architecture and conformation of adhesion molecules could also alter the efficiency of nucleotide delivery. The effect of the adhesion ligand conformation has not been examined in this context, but it does modulate other aspects of cell signalling[115]. The architecture of the cell culture has been noted to play a critical role in the delivery of nucleotides, for example, therapeutic effects of nucleotides in 3D tumour spheroids are localized to dividing cells at the periphery of the spheroids[32]. This finding suggests that it may be problematic to predict *in vivo* effects from the standard 2D culture systems commonly used for *in vitro* studies. The efficiency of gene transfer may also be enhanced by physically immobilizing DNA complexes onto surfaces that also support cell adhesion[116], presumably by concentrating the DNA at the cell surface.

The mechanical properties of the ECM and the dynamic mechanical loading of cells adhered to a synthetic ECM may be crucial regulators of the uptake and expression of pDNA. The stiffness of the ECM has specifically been found to alter the expression of pDNA, probably through the regulation of cell proliferation[67]. Increasing the elastic modulus of a model ECM led to a higher efficiency of gene transfer and expression in cells[117] (FIG. 4c), and this role of matrix stiffness in gene delivery was also found with cells in 3D culture. Similar results have been noted with cell cultures in collagen gel matrices that are mechanically reinforced with poly(glycolic acid) fibres[118]. External mechanical stimulation may also mediate gene delivery by altering the concentration gradient of nucleotides around cells and directly modulating the cellular phenotypes. For example, endothelial cells that are exposed to a convective flow of cell-culture medium exhibited a higher efficiency of nucleotide uptake and expression than cells cultured under static conditions, due to an enhanced supply of nucleotides to the cells and increased cell growth[34]. It is likely that other types of mechanical stimulation (for example, tensile and compressive stresses and strains) will also influence the ability of a cell to take up nucleotides[44,119–121]. Because of the multiple potential mechanisms by which the ECM mechanical properties and mechanical stimulation alter the microenvironment (for example, altered mass transport), the relationship of the mechanical effects to nucleotide therapy efficiency will need to be carefully examined. Furthermore, the effects of static and dynamic mechanical signals may be compensated or amplified by other aspects of the cellular microenvironment, including the spatial organization of cell-adhesion molecules and concentration of exogenous soluble factors.

The *in vitro* cellular microenvironment is also likely to be vital in the quality of *ex vivo* cell transfection[122–124] and *de novo* tissue generation using genetically engineered cells (for example, skeletal muscle, skin graft)[125]. These methods have been developed as alternative ways to deliver therapeutic proteins *in vivo* over extended periods of time[126,127], and the natural and synthetic biomaterials used as a temporary ECM in these approaches will probably affect their utility.

The importance of the cellular microenvironment in the success of oligonucleotide and siRNA therapies has not been studied so far to our knowledge. However, the development of these therapies is following a similar path to pDNA approaches (for example, complexation with polycations to enhance the passage of pDNA across the cell membrane[105]), and it is likely that aspects of the cellular microenvironment that regulate the cellular response to pDNA, will also modulate the cellular response to oligonucleotides and siRNA. These *in vitro* studies have motivated several strategies aimed at improving *in vivo* therapeutic efficiency of nucleotides, as described in the next section.

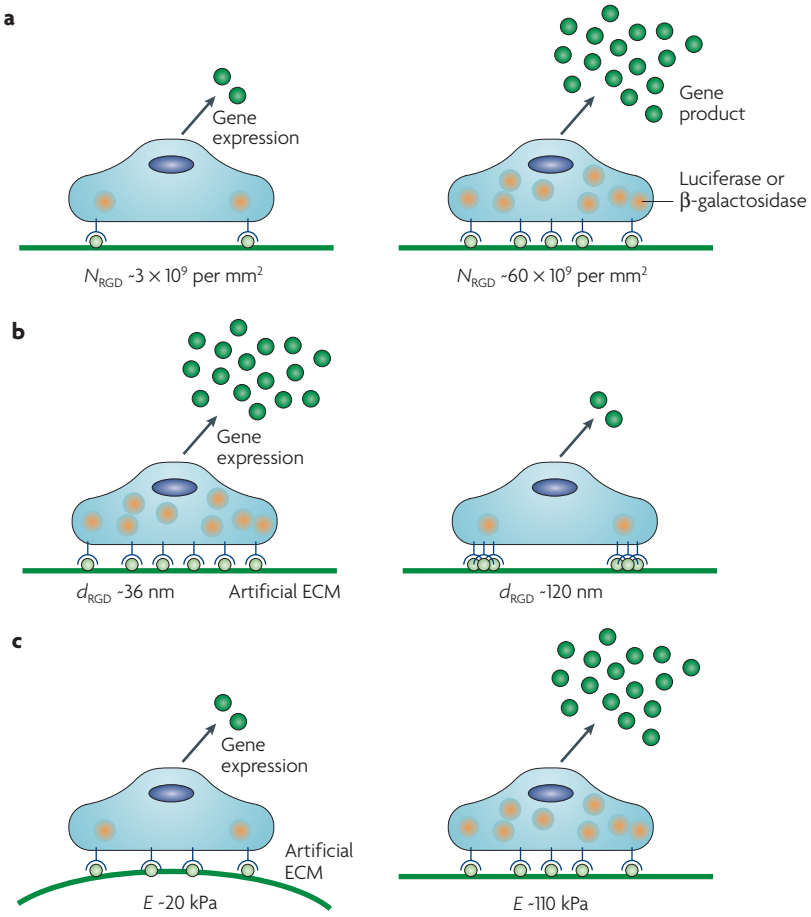

Figure 4 | ***In vitro* efficiency of gene transfer and subsequent level of expression are modulated by properties of the extracellular matrix (ECM).** The ability of a cell to take up and express plasmid DNA encoding luciferase or β-galactocidase and to subsequently express the gene product was enhanced by increasing the total number of cell-adhesion oligopeptides (N_{RGD}) coupled to substrates (**a**), and decreasing the nanoscale spacing between clusters of cell-adhesion oligopeptides (d_{RGD})[114] (**b**), or increasing the stiffness of the adhesion substrates that are presenting the oligopeptides (**c**)[117].

Strategies to engineer the cell microenvironment and to manipulate cell responsiveness. Nucleotides are often delivered via injection of a solution (for example, intramuscular or intravenous injection)[128]; however, the transport of the nucleotide may be impeded by a damaged ECM and its uptake limited by the intrinsic internalization ability of the cell. It might, similar to protein therapies, be crucial to engineer the cellular microenvironment of the target tissue to maximize the *in vivo* therapeutic efficiency of nucleotides, and similar strategies as those proposed for protein drugs could be useful.

The results of the limited studies so far suggest that a number of supplemental factors can potentially be useful as co-drugs with nucleotides to enhance cell proliferation, and indirectly increase nucleotide uptake. Co-delivery of bFGF with pDNA has been demonstrated to increase the level and duration of gene expression of marker genes. This approach may potentially increase the effect of therapeutic protein expression[129]. Because growth-factor receptors are spatially associated with integrins at the focal-adhesion complex[130], the binding of bFGF might have multiple effects. The inclusion of a pDNA encoding dendritic-cell growth factor and fms-related tyrosine kinase 3 (FLT3) ligand in a DNA vaccine similarly enhanced the level of the resultant immune response[131]. These effects of supplemental factors may be highly dependent on the delivery sequence (for example, sequential versus simultaneous delivery).

Interactions between cells and the ECM can also be targeted through the modulation of integrin expression to alter nucleotide effectiveness. TGFβ has been used to enhance the delivery of non-viral gene vectors, which target α5β1 and α5β3 integrins, as TGFβ leads to the overexpression of these integrins[132]. Conversely, overexpression of integrins in pathologic tissue may limit the effectiveness of certain oligonucleotide and siRNA drugs, and downregulating integrin expression in the target cells may maximize the efficiency of these therapies.

Implantation of a nucleotide-releasing engineered ECM may significantly enhance the efficiency of certain nucleotide therapies. Porous synthetic matrices (for example, poly(lactide-co-glycolide)[133] and gene-activated matrices[134]) used for the sustained and localized delivery of pDNA have been implanted into tissue defects to facilitate cell migration from peripheral tissue and to enhance the subsequent tissue regeneration[135]. Combining multiple elements of the ECM, such as a biomineral with collagen, in the DNA encapsulation matrices may further enhance gene-transfection efficiency and subsequently promote regeneration owing to the multiple functions of the matrix components[136]. Although it has not been systematically examined, presentation of cell-adhesion molecules and the mechanical properties of the nucleotide-releasing ECM could modulate *in vivo* therapeutic efficiency, and external stimulation may also be useful for improving the *in vivo* uptake of nucleotides by cells. These variables of the cellular microenvironment are also likely to be crucial for the function of cells transfected *ex vivo* on a material carrier and subsequently transplanted[137].

Conclusions and future directions

The microenvironment of the cell plays a critical role in mediating the cellular response to exogenous growth factors and nucleotides that are delivered for local therapies. The microenvironment may also be altered because of disease and injury. Both stimulatory and inhibitory effects of proteins and nucleotides can be either amplified or offset depending on the cell's adhesion to the ECM, the chemical structure of the cell adhesion cues, the physical properties and architecture of the ECM, and the presence of supplemental molecules that upregulate or downregulate adhesion-receptor expression. However, there have been few efforts so far that aim to engineer the microenvironment and alter the cellular response to biomacromolecular therapies. Therefore, it might be fruitful to translate, in a careful manner, results from *in vitro* studies to the clinical settings to improve the current performance of biomacromolecular therapies. Specifically, parallel control of several variables of the cellular microenvironment (for example, the use of

factors to regulate integrin expression) and therapeutic biomacromolecules (for example, molecular structure and delivery strategy) may provide synergistic improvements of therapies.

Biomacromolecular therapies that aim to regenerate tissues may be enhanced by providing an artificial ECM designed to provide migratory or transplanted cells with a biochemical and biophysical microenvironment appropriate to elicit desirable cell signalling[93,138]. As demonstrated with *in vitro* studies, several variables of the artificial ECM play a role in regulating the cellular response to therapeutic proteins and nucleotides. Incorporation of these variables into the design of ECMs, which also function as a depot of protein and nucleotides, can promote cell proliferation, migration, and subsequent tissue regeneration. Various nanoscale and microscale techniques[95,139,140] will probably provide greater benefits in modulating properties of the artificial ECM, and thus allow one to achieve significant advances in the success of biomacromolecular therapies.

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Competing interests statement
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